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(54) **CAPILLARY ELECTROPHORETIC METHOD TO DETECT TARGET-BINDING LIGANDS AND TO DETERMINE THEIR RELATIVE AFFINITIES**

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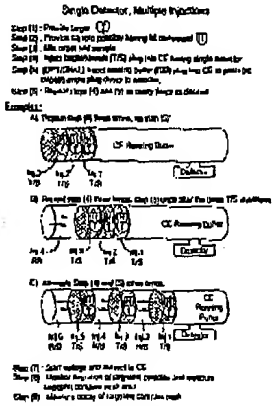
(57) **ABSTRACT**

The invention relates to capillary electrophoretic methods for detecting ligands or hit compounds that can bind to a selected target at or above a selected binding strength. The method allows one to rank various ligands based on their relative affinity, i.e., the relative stability of the target/ligand complex during capillary electrophoresis under selected conditions. The method also enables selective detection of strong-to-moderate binding hit compounds, even in the presence of high concentrations of weaker, competitive hit compounds.

28 Claims, 7 Drawing Sheets



CAPILLARY ELECTROPHORETIC METHOD TO DETECT TARGET-BINDING LIGANDS AND TO DETERMINE THEIR RELATIVE AFFINITIES

This application claims the benefit of Provisional application Ser. No. 60/068,781, filed Dec. 24, 1997.

FIELD OF THE INVENTION

This invention relates generally to the discovery of new regulatory compounds, drugs, and/or diagnostic agents. In particular, it relates to capillary electrophoretic methods for detecting ligands binding to a known target molecule, and for determining the relative stabilities of ligand/target complexes, thereby allowing the ranking of different ligands according to their relative binding strengths to a common target. The invention encompasses methods of detecting moderate-to-strong binding ligands in mixtures that also have much higher concentrations of competing, weaker-binding ligands.

BACKGROUND OF THE INVENTION

Developing screens to identify new biologically active compounds can present unique and difficult challenges, especially when screening complex materials, particularly "complex biological materials": any material that may have an effect in a biological system. Examples include, but are not limited to: naturally occurring samples, products or extracts; various biological preparations; chemical mixtures; libraries of pure compounds; and combinatorial libraries. Examples of major screening problems include: detecting potential hit compounds that bind to a target of interest, especially ligands present at low concentrations in screened samples; accounting for unknown components that can interfere with screening agents; and determining the relative value of screened samples for further investigative efforts. As well, high concentrations of a weak or several weak, competing binder(s) can mask the signal from a moderate-to-strong hit compound occurring at a lower concentration in the same sample.

Recently, the use of capillary electrophoresis techniques has facilitated and improved the process of screening for unknown, biologically active compounds. For example, WO 97/22000 encompasses four broad embodiments of a capillary electrophoretic screening method, as follows.

(1) In a non-competitive embodiment of WO 97/22000, a target and complex biological sample are mixed together, then an aliquot of that target/sample mixture is subjected to capillary electrophoresis (CE), and the CE migration of the target is tracked. The target's migration pattern under these conditions are compared against a reference standard, typically the unbound target's migration pattern in the absence of any target-binding ligand.

(2) In a non-competitive, subtractive analysis embodiment of WO 97/22000, a target and sample are mixed together and then subjected to CE. The migration pattern of this mixture is compared to the migration pattern of a sample of the complex biological material alone. Any difference between the two migration patterns suggests the presence in the sample of a hit compound that can bind to the target.

(3) One competitive binding embodiment is provided in WO 97/22000, which tracks known, charged ligand: The target is first mixed with a complex biological material sample, and then with a known, charged ligand that binds tightly to the target, to form a sample/target/known ligand mixture. This method uses an essentially equilibrium setting when incubating target and known, tight-binding ligand together, so that the known, tight-binding ligand can displace any weaker-binding hit, prior to CE. This mixture is subjected to capillary electrophoresis and the migration of the known, charged ligand is tracked. (Thus, this method is useful when the target is not easily detected during CE.) Any difference in the known, charged ligand's migration pattern, when in the presence of both the target and a complex biological material sample, from the known ligand's migration pattern when in the presence of the target alone, indicates the presence of a candidate, unidentified target-binding ligand in that sample.

(4) In another competitive binding embodiment of WO 97/22000, the target's migration is tracked and the CE running buffer contains a known, weak-binding, competitive ligand. The target is mixed with a sample, and an aliquot of the mixture is subjected to CE in the presence of a known, relatively weak, target-binding 'competitor' ligand in the CE running buffer. The migration of the target is tracked during CE. The reference standard is the migration of a target plug alone in the known ligand-containing CE buffer, its migration being shifted by its weak, reversible binding to the known ligand dispersed in the CE buffer, as compared to the target's migration alone ligand-free buffer. This competitive screening method can detect a tight-binding hit compound in a target/natural sample mixture, because the hit binds up the target for the entire CE run and prevents the target's interaction with the known weak-binding ligand in the buffer. Therefore, the CE migration pattern of the target in the sample/target aliquot would shift back to the target's migration position as it would be in ligand-free running buffer. This method, too, is particularly useful when the unbound target is not easily detected in ligand-free buffer during CE.

While WO 97/22000 provides useful CE screening methods, they do not completely solve the screening problems listed previously.

Therefore, there remains a need for rapid and cost-effective screening tools for discovering new bioactive compounds and potential regulatory compounds that bind to essential molecules of key metabolic pathways. Also needed is a way of prioritizing candidate ligands and samples of material for further characterization. The present invention addresses these needs, by providing: a means of detecting unknown ligands that may be candidate, new, bioactive compounds; a means of ranking screened samples detected to contain candidate hit compounds or ligands, according to their relative binding strengths and value as potential sources of regulatory and diagnostic compounds; and a means of identifying effective and valuable, strong or moderate, target-binding ligands in the presence of weaker, competitive binders. Identifying and ranking those ligand-containing samples that form the most stable complexes with the selected target, saves time and resources spent on further isolation and characterization of hit compounds. The most stable ligands are potentially more effective and valuable as therapeutic, regulatory and/or diagnostic compounds and drugs.

SUMMARY OF THE INVENTION

The present invention provides: (1) a means to screen for target-binding ligands of a desired binding strength, in complex biological and other materials and mixtures; (2) a means to screen for the ligands or hit compounds of the desired affinity, even in the presence of weak-binding ligands in mixtures; and (3) a means to rank hit compounds according to their relative affinities. All aspects of the

able to effect various changes, substitutions of equivalents, and other modifications to the methods and compositions set forth herein.

What is claimed is:

1. A method to detect, in a sample of complex biological material, any candidate hit compound that binds to a selected target at or above a selected binding strength and to determine a stability, during capillary electrophoresis, of any target/hit compound complex detected, wherein the stability is determined to identify a relative affinity of a hit compound, said method comprising, in the order given, the steps of:

(1) providing a predetermined concentration of a selected target;

(2) providing a sample of complex biological material;

(3) mixing the target with the sample to form a target/sample mixture and incubating the target/sample mixture;

(4) injecting an aliquot of the target/sample mixture into a capillary electrophoresis instrument comprising a capillary having a single detection point;

(5) subjecting the target/sample aliquot to capillary electrophoresis under a set of conditions to detect any hit compound in the sample that binds to the target at or above a selected binding strength;

(6) tracking a capillary electrophoretic migration of the target in the injected target/sample aliquot at the detection point in the capillary electrophoresis instrument;

(7) repeating steps (4)–(6) one or more times, wherein each performance of steps (5)–(6) uses a different set of conditions for a different selected binding strength,

wherein each injected target/sample aliquot undergoes a different capillary electrophoresis run time from other injected target/sample aliquot(s), thereby generating multiple capillary electrophoretic profiles, of the same target/sample aliquot,

wherein the different conditions are optimized so that any complex, between the target and any candidate hit compound at or above each selected binding strength, generates a different result in tracking the capillary electrophoretic migration as in step (6);

(8) determining whether at least one capillary electrophoretic profile of the target generated from step (6), when compared to a reference standard, indicates a presence of any hit compound(s) binding to the target; and

(9) determining the stability, during capillary electrophoresis, of any target/hit compound complex(es) detected, by comparing the multiple capillary electrophoretic profiles.

2. The method of claim 1, each repeat of step (4) comprising pushing each injected target/sample aliquot to a different starting point in the capillary prior to capillary electrophoresis, by pressure-injecting an aliquot of capillary electrophoresis running buffer into the capillary behind the injected target/sample aliquot, wherein the running buffer aliquot differs in amount for each repeat of step (4).

3. A method to detect, in a sample of complex biological material, any candidate hit compound that binds to a selected target at or above a selected binding strength and to determine a stability, during capillary electrophoresis, of any target/hit compound, complex detected, wherein the stability is determined to identify relative affinities of hit compounds, said method comprising, in the order given, the steps of:

(1) providing a predetermined concentration of a selected target;

(2) providing a sample of complex biological material;

(3) mixing the target with the sample to form a target/sample mixture and incubating the target/sample mixture;

(4) sequentially injecting multiple aliquots of the target/sample mixture into a capillary electrophoresis instrument comprising a capillary having a single detection point, such that the multiple target/sample aliquots are spaced apart along an axis of the capillary;

(5) subjecting the multiple target/sample aliquots within the capillary simultaneously to capillary electrophoresis, under conditions to detect any hit compound in the sample that binds to the target, so that a detectable amount of any complex formed in the multiple target/sample aliquots between the target and any candidate hit binding to the target at or above a selected binding strength of each target/sample aliquot undergoes a different capillary electrophoresis run time before reaching the detection point;

(6) tracking, at the detection point, a capillary electrophoretic migration of the target in each of the multiple target/sample aliquots, thereby generating different multiple capillary electrophoretic profiles of the target/sample aliquot at different capillary electrophoretic run times;

(7) determining whether at least one capillary electrophoretic profile of the target generated from step (6), when compared to a reference standard, indicates a presence of any hit compound(s) binding to the target; and

(8) determining the stability, during capillary electrophoresis, of any target/hit compound complex(es) detected during step (6), by comparing the multiple capillary electrophoretic profiles.

4. The method of claim 3, step (4) further comprising pressure-injecting an aliquot of capillary electrophoresis running buffer into the capillary behind each injected target/sample aliquot, to place each injected target/sample aliquot at a different starting point in the capillary, prior to the capillary electrophoresis step.

5. The method of claim 3 or 4, wherein an aliquot of capillary electrophoresis running buffer is pressure-injected into the capillary immediately behind a last injected target/sample aliquot, prior to capillary electrophoresis, to push the last injected target/sample aliquot to a desired starting point in the capillary.

6. A method to detect, in a sample of complex biological material, any candidate hit compound that binds to a selected target at or above a selected binding strength and to determine a stability, during capillary electrophoresis, of any target/hit compound complex detected, wherein the stability is determined to identify relative affinities of hit compounds, said method comprising, in the order given, the steps of:

(1) providing a predetermined concentration of a selected target;

(2) providing a sample of complex biological material;

(3) mixing the target with the sample to form a target/sample mixture and incubating the target/sample mixture;

(4) injecting an aliquot of the target/sample mixture into a capillary electrophoresis instrument comprising a capillary having multiple detection points;

(5) subjecting the target/sample aliquot to capillary electrophoresis under conditions to detect a hit compound